

Translocation of Carbon from Surface Organic Horizons to the Subsoil in Coarse-Textured Spodosols: Implications for Deep Soil C Dynamics

David E. Rothstein*

Dep. of Forestry
Michigan State Univ.
East Lansing, MI 48824

Ehsan R. Toosi

Dep. of Plant, Soil & Microbial Sciences
Michigan State Univ.
East Lansing, MI 48824

Randall J. Schaetzl

Dep. of Geography
Michigan State Univ.
East Lansing, MI 48824

A. Stuart Grandy

Dep. of Natural Resour. & Environment
Univ. of New Hampshire
Durham, NH 03824

Dissolved organic matter (DOM) translocated from O horizons has been viewed as the main source of subsoil carbon (C) in Spodosols. However, recent studies in other soil types have concluded that little O horizon DOM reaches the subsoil directly. We investigated whether C derived from O horizons contributes to deep-soil C stores in six Spodosol profiles under coniferous (red pine) or deciduous (sugar maple) forests. We used spectroscopic, stable-isotope and pyrolysis-gas chromatography/mass spectroscopy analysis of DOM and solid phase soil samples to assess whether O horizon C was transported directly to deep soil horizons or had been exchanged with mineral soil organic matter (SOM) pools. All three approaches indicated that O horizons are the source of the majority of DOM entering B horizons, with little contribution from desorbed SOM. Despite a greater stock of O horizon C in red pine stands, there was no difference between forest types in the quantity of C delivered to B horizons ($18 \text{ g C m}^{-2} \text{ y}^{-1}$). However, C derived from O horizons accounted for ~95% of the C entering B horizons in sugar maple stands, but only ~80% in red pine stands. Molecular analysis of DOM and SOM also showed a greater resemblance of B horizon SOM to DOM in sugar maple stands; however, it also clearly indicated the importance of microbial processing of input C in the formation of B horizon SOM. Our results demonstrate the strong connection between O horizons and deep soil C stocks in these sandy Spodosols and indicate that future changes in the quantity or quality of DOM entering the mineral soil have the potential to alter the delivery of surface C to depth.

Abbreviations: DOM, dissolved organic matter; ITM, imogolite-type minerals; SOM, soil organic matter.

Worldwide, deep soil horizons (>20 cm depth) represent a tremendous reservoir of stored carbon (C; ~1700 Pg) (Jobbagy and Jackson, 2000). Despite this, the properties and dynamics of C stored in sub-surface horizons are poorly understood (Harrison et al., 2011; Rumpel and Kogel-Knabner, 2011). Dissolved organic matter (DOM) originating from surface O horizons has traditionally been viewed as a major source of organic C in the subsoil (Kalbitz et al., 2005; Sayer, 2006). This is particularly the case for Spodosols (Podzols), where years of research have focused on the transport of O horizon C to depth as the dominant pedogenic process (Lundström et al., 2000; Sauer et al., 2007). In this model, DOM generated in O horizons is transported by percolating water through the E horizon, where it is deposited at depth, building organic C stocks in the B horizon below.

On the other hand, several recent studies, using isotopically labeled litter additions (Hagedorn et al., 2002, 2004, 2012; Fröberg et al., 2007; Kramer et al., 2010), have concluded that, in some soils, little of the DOM solubilized from sur-

Core Ideas

- O horizons account for the majority of DOM entering B horizons in these sandy soils.
- There was less exchange with SOM in sugar maple stands compared to red pine stands.
- Microbial processing of DOM inputs appears to be important in the formation of SOM.

Soil Sci. Soc. Am. J. 82:969–982

doi:10.2136/sssaj2018.01.0033

Received 18 Jan. 2018.

Accepted 30 Mar. 2018.

*Corresponding author (rothste2@msu.edu).

© Soil Science Society of America, 5585 Guilford Rd., Madison WI 53711 USA. All Rights reserved.

face litter reaches the subsoil directly, implying that O horizon C is largely “disconnected” from mineral soil C stocks (Garten, 2009). These findings, along with other work investigating the contribution of root turnover to SOM (Rasse et al., 2005; Bird and Torn, 2006) and the contribution of microbial products to SOM (Miltner et al., 2012; Gleixner, 2013; Kallenbach et al., 2016), have led to the prevailing view that in situ root production and decay and microbial products represent the main source of C in mineral soils, with little direct contribution from O-horizon derived DOM. The apparent disconnect between surface litter C and mineral soil C, and the predominance of roots as a source of mineral soil C, has even made its way as a general principle into the literature on management of forests for C sequestration (Price et al., 2012).

This emerging view of a disconnect between surface O horizons and the subsoil is at odds with decades of research on Spodosol genesis, which have consistently concluded that C-rich Bhs horizons result from downward percolation of DOM derived from the O horizon, (Lundström et al., 2000; Sauer et al., 2007). Furthermore, in their conceptual review of soil DOM dynamics, Kaiser and Kalbitz (2012) argued that rapid exchange between DOM and SOM should be reduced under conditions of low SOM, low reactive mineral contents and fast water movement. Thus Spodosols, with a sandy upper solum (E horizon) with low SOM and stripped of reactive minerals should represent ideal conditions for the direct delivery of surface C to depth.

In addition to physical and chemical properties of the mineral phase, the chemical composition of input DOM also can have a strong influence on the degree of exchange between DOM and SOM as water percolates through soil. Specifically, hydrophobic, lignin-derived compounds in DOM appear to preferentially sorb and displace more hydrophilic compounds from soil reactive surfaces (Kaiser and Guggenberger, 2000; Kaiser and Kalbitz, 2012; Scott and Rothstein, 2014). This suggests that differences in litter chemistry among forest cover types, particularly with respect to litter lignin content, may also influence the delivery of surface C to depth.

We investigated the potential for O horizon C to contribute to deep-soil C stores in six well-developed Spodosol pedons in northern Michigan, USA. Three of the soils are under deciduous angiosperm (sugar maple; *Acer saccharum* Marsh.) forest, while the other three are beneath evergreen conifer (red pine; *Pinus resinosa* Aiton) plantation forest. We hypothesized that C derived from O horizons should make a greater contribution to DOM entering the B horizon in these coarse-textured Spodosols, when compared to what has been recently reported for other soil types. We further hypothesized that the exchange of O-horizon DOM with SOM would be greater in red pine stands because of the higher lignin content of red pine litter compared to sugar maple litter (Aber et al., 1990). Finally, we hypothesized that the molecular composition of B horizon SOM would bear a strong resemblance to O horizon DOM, indicative of surface organic horizons as the main source of subsoil C.

METHODS AND MATERIALS

Study Sites, Soil Characterization, and Instrumentation

We selected six study sites in the Upper Peninsula of Michigan by first developing a list of candidate stands in a Geographic Information System (GIS) based on vegetation (sugar maple or red pine as the dominant overstory species), stand age (≥ 50 yr old), soil parent material (sandy glacial outwash), and soil classification (well-drained Haplorthods and Durorthods). We developed an initial list of 30 red pine and 17 sugar maple stands that met these criteria within ~ 10 km of each other. Potential stands were then visited and eliminated if: (i) the parent material was not outwash, (ii) the stand showed evidence of recent disturbance, especially harvesting, or (iii) the soil had been furrowed during planting of red pine. The final six sites (3 red pine and 3 sugar maple) were located within an approximately 17-km² area on a sandy upland in the eastern Upper Peninsula of Michigan. Pre-settlement vegetation at our study sites consisted of beech (*Fagus grandifolia* Ehrh.)–sugar maple–hemlock [*Tsuga canadensis* (L.) Carrière] forest, which was harvested during the late 19th to early 20th century (Comer et al., 1995). Sites currently assigned to our sugar maple treatment appear to have regenerated naturally following logging, whereas sites assigned to our red pine treatment appear to have experienced severe post-logging fire and were replanted to red pine as part of reforestation efforts of the 1930s.

At each site we located study plots on level ground, with minimal disturbance from tree uprooting. At each plot we excavated a 6-m long by 1.8-m deep trench, and a cleaned pit face was described and sampled by the standard methods (Schoeneberger et al., 2012). Typical horizon sequences were Oi, Oe, E, Bhs, Bs1, Bs2, and Bw at sugar maple sites and Oi, Oe, E, Bs1, Bs2, Bs3, and Bw at red pine sites. O horizon samples were collected using a 30-cm by 30-cm sampling frame, whereas mineral soil samples were collected by horizon from the pit face. Bulk density samples for each mineral soil horizon were taken using a thin-walled aluminum ring, of 10-cm diameter, driven into the pit face. Fine roots (< 2 mm diameter) were sampled by collecting three 5.1-cm diameter cores from the top of the Oe to 50-cm depth into the mineral soil, which were sectioned into 10-cm increments. Fine roots were hand-picked from the Oe horizon and each 10-cm mineral soil increment, washed on a 0.5-mm sieve and oven dried at 65°C.

All soil samples were air dried and lightly ground to pass a 2-mm sieve. Subsamples of all mineral and organic soil horizons were pulverized in a ball mill and analyzed for C concentration by dry combustion gas chromatography on a Costech ECS 4010 (Costech Analytical Technologies Inc., Valencia, CA). Soil texture was determined on organic matter-free (H₂O₂), dispersed [(NaPO₃)₁₃×Na₂O] samples using a Malvern Mastersizer 2000E laser particle size analyzer (Miller and Schaeztl, 2012). Iron (Fe), aluminum (Al), and silicon (Si) extractions were conducted on all mineral soil samples, using traditional extractants of sodium citrate-dithionite, Na-pyrophosphate, and acid ammonium oxalate (McKeague, 1967) and analyzed by inductively-coupled plasma, atomic emission spectrometry (Optima 2100

Table 1. Vegetation and soil characteristics of the red pine (RP, 1–3) and sugar maple (SM, 1–3) study sites.

	RP1	RP2	RP3	SM1	SM2	SM3
Latitude (N)	46.33	46.32	46.31	46.32	46.32	46.32
Longitude(W)	85.02	85.02	85.02	85.04	85.06	85.06
Contemporary vegetation						
Stand basal area, m ² ha ⁻¹ †	47.5	42.9	38.3	36.0	33.7	32.1
Relative dominance, %‡						
<i>Pinus resinosa</i>	98.4	91.1	98.0	0	0	0
<i>Pinus strobus</i>	1.6	8.9	2.0	0	0	0
<i>Acer saccharum</i>	0	0	0	100	84.1	66.7
<i>Acer rubrum</i>	0	0	0	0	0	23.8
<i>Fagus grandifolia</i>	0	0	0	0	4.5	2.4
<i>Prunus serotina</i>	0	0	0	0	9.1	7.1
<i>Betula alleghaniensis</i>	0	0	0	0	2.3	0
Stand age (in 2012)‡	78	78	75	98	97	97
Soil property						
O horizon C stock, kg m ⁻²	2.5	2.3	2.0	1.0	1.0	0.5
E horizon C, g kg ⁻¹	16.8	5.8	3.7	4.0	1.4	1.7
Uppermost B horizon C, g kg ⁻¹	20.6	9.4	18.6	11.0	11.1	9.6
Second B horizon C, g kg ⁻¹	8.2	8.8	8.9	7.5	7.5	7.4
E horizon bulk density, g cm ⁻³	1.18	1.23	1.36	1.31	1.28	1.26
E horizon texture, % silt+clay	12.1	10.3	11.3	11.3	11.0	9.5
B Horiz. Texture (% silt+clay)§	4.5	7.2	7.4	9.8	7.1	4.4
E horizon ITM, %§	0.0066	0.0061	0.0054	0.0016	0.0003	0.0067
B horizon ITM, %§	0.85	0.58	1.24	0.47	0.40	0.58
Second lysimeter depth, cm¶	10	17	11	8	26	17
Third lysimeter depth, cm¶	43	55	55	53	50	70

† Determined in the field with 10 Basal Area Factor angle gauge at three points per stand.

‡ From USDA-Forest Service Stand Inventory Database.

§ IMT, imogolite-type minerals; B-horizon texture and percentage of ITM is a depth-weighted average of the two uppermost B horizons (i.e., those directly above the lowermost lysimeter).

¶ Installation depth of each lysimeter, relative to the top of the mineral soil. The second lysimeter depth corresponds to the bottom of the E horizon; the third lysimeter depth corresponds to the bottom of the B horizon (usually Bs1 or Bs2).

DV, PerkinElmer, Bridgeport, CT). For this study we calculated the amount of imogolite-type minerals (ITM), an important phase of short-range order minerals, using the ammonium-oxalate extractable Si and the calculation formula of Mizota and Van Reeuwijk (1989), assuming a molar ratio of Al to Si(X) of 2. Initial vegetation and soil characteristics for all six study sites are reported in Table 1 and fine root biomass by depth in Table 2.

One zero-tension lysimeter was installed at the base of the O, E, and second B horizons in each plot (Fig. 1). Thus our first lysimeter captured water leaving the O horizon and entering the mineral soil, our second lysimeter captured water entering the B horizon and our third lysimeter captured water draining from the lower B horizon. Average depths for the E and B horizon lysimeters were 15 and 54 cm, respectively (Table 1). The lysimeters were constructed using 16-cm diameter HDPE funnels filled

with combusted (550°C), acid-washed (10% HCl) quartz sand, as described by Wilson et al. (1995) and MacDonald et al. (2008). This type of lysimeter is suitable for collecting soil solution in fast flowpaths (e.g., Weihermüller et al., 2007) the dominant form of soil solution in coarse-textured and unstructured soils. Lysimeters were installed at staggered horizontal locations along the pit face. Funnels were supported with custom-made ABS plastic holders, outfitted with turnbuckles that were used to raise the lysimeters into contact with undisturbed soil above (Fig. 1).

Table 2. Fine root (<2-mm diameter) biomass by depth for red pine and sugar maple stands. Values are means ± 1 SE of three stands per ecosystem type.

Soil depth	Red pine	Sugar maple
	Fine root biomass	
	g m ⁻²	
Oe horizon	432 ± 23.0	255 ± 57.6
0–10 cm	221 ± 42.8	180 ± 42.2
10–20 cm	155 ± 31.7	216 ± 25.9
20–30 cm	104 ± 11.1	130 ± 6.1
30–40 cm	42 ± 9.6	90 ± 31.6
40–50 cm	20 ± 10.7	18 ± 0.6

**Fig. 1. Photograph showing the location and installation of zero-tension lysimeters below the Oe horizon (right), E horizon (middle) and lower B horizon (left).**

Lysimeter Sampling and C Flux Measurements

The six plots were instrumented in May 2012, and lysimeters first collected on 28 June 2012. After that, we monitored US National Weather Service projections of precipitation and snowmelt (<https://www.noahrs.noaa.gov/nsa/>) and traveled to the sites to collect lysimeter samples after rain or snowmelt events of 2 cm or more water equivalency. The one exception to this sampling approach was during the ~4 wk period of nearly continuous melting of the snowpack that occurred each spring. During this period we visited sites and cleared lysimeters weekly until all snow was gone from our plots. Samples were collected in HDPE bottles and kept on ice for transport back to the laboratory, where they were filtered (0.45 μ M Millex MCE Membrane; Merck Millipore Inc.; Cork, IRL) and then frozen (-20°C) for future analyses. To provide for an equilibration period following the disturbance associated with plot instrumentation, we discarded the water collected between June and September, 2012. Here we report data for two full water-years- from 1 Oct. 2012 through 30 Sept. 2014, encompassing 36 individual sampling dates. We analyzed every lysimeter sample collected for dissolved organic C (DOC) by oxidative combustion-infrared analysis (Shimadzu TOC-VCNP, Shimadzu Corp., Kyoto, Japan).

We used the BROOK90 forest hydrological model (Federer, 2013) to estimate the volume of water percolating through the soil. We supplied the model with daily precipitation and temperature data from a nearby National Weather Service Station (Newberry, MI; station ID USC00205816) and parameterized the model separately for each of our six sites based on soil texture, horizon depths and vegetation type (evergreen conifer vs. broadleaved deciduous). To determine DOC fluxes over each sampling interval, we summed the daily estimates of water flux at each depth over the collection period and multiplied that by the DOC concentration of the sample.

We collected 512 lysimeter samples over 36 sampling dates between 1 Oct. 2012 and 30 Sept. 2014. If all lysimeters had produced sample on every sample date, we would have had 648 samples. When a lysimeter was empty (dry) but BROOK90 estimated water flux over that period, we gap-filled DOC concentration data by averaging values from the most recent sample dates before and after. Because empty lysimeters occurred primarily during dry summer months when little water was moving through the profile, we expected that gap filling of concentrations would have relatively little influence on DOC flux estimations. To test this, we separately calculated cumulative DOC fluxes using only dates for which we collected samples. Across the 2-yr study, sample dates with directly-measured DOC concentrations accounted for 98, 99, and 92% of our total estimated flux from the O, E and B horizons in sugar maple stands and 97, 96, and 79% in red pine stands.

DOM and SOM Chemistry

We conducted detailed analyses of DOM chemistry on samples collected on six dates (21 Oct. and 12 Nov. 2012, and 27 Apr., 23 July, 30 July, and 26 Aug. 2013) using isotopic and spectroscopic methods. We selected these dates based on two criteria.

First, because they had the most complete representation of samples with almost all of the lysimeters yielding samples. Second, because they were well distributed across the year, capturing the three distinct periods of hydrologic flux we had described previously (Schaeztl et al., 2015): spring snowmelt (27 Apr. 2013), summertime storms (23 July, 30 July, and 26 Aug. 2013), and post leaf-senescence rains (21 Oct. and 12 Nov. 2012).

We measured ultraviolet absorption of lysimeter samples at 254 nm on an ATI Unicam UV/Vis Spectrophotometer (ThermoSpectronic, Rochester, NY) using a 1-cm quartz cuvette and report data as specific UV absorbance (SUVA_{254}), normalized for the DOC concentration in each sample (Weishaar et al., 2003). The natural abundance $\delta^{13}\text{C}$ of DOC (hereafter referred to as DO^{13}C) was determined using an O.I. Analytical Model 1030 TOC Analyzer (OI Analytical, College Station, TX) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK) at the University of California, Davis' Stable Isotope Facility. Values of $\delta^{13}\text{C}$ are expressed with respect to Vienna Pee Dee Belemnite.

The molecular chemistry of DOM was determined using pyrolysis-gas chromatography/mass spectroscopy (py-GC/MS) of lyophilized samples collected during the spring 2013 snowmelt period. To generate the minimum of 3 mg of total C needed for py-GC/MS analysis we pooled O- and E-horizon solutions from across three sample dates (19 Apr., 27 Apr., and 4 May 2013), prior to freeze-drying. C concentrations in B horizon lysimeter samples were so low that we were forced to pool across the same three sample dates, and across our three sites, yielding only one sample for each ecosystem type.

To better understand the potential contribution of surface-litter derived DOM to subsoil C stocks, compared to that of other C sources, we also analyzed solid samples of fine roots (composited across depths), Oi, Oe, E and the uppermost B horizons (Bhs or Bs1) by py-GC/MS. Lyophilized DOM samples and solid samples were pulse-pyrolyzed at 600°C on a Pyroprobe 5150 (CDS Analytical Inc., Oxford, PA), transferred to a gas chromatograph (Trace GC Ultra, Thermo Scientific) for pyrolysis-product separation and then transferred to a mass spectrometer (Polaris Q, Thermo Scientific) for compound identification. Mass spectrometer peaks were identified using the Automated Spectral Deconvolution and Identification System (AMDIS V 2.65, National Institute of Standards and Technology, Gaithersburg, MD) and the mass spectral compound library from the National Institute of Standards and Technology. Individual compounds were expressed as a relative abundance of the total identifiable peak area and grouped into the following classes based on their origin: lignin-derived compounds (lignin subunits and their derivatives), other aromatics, lipids, proteins, other N-bearing compounds, phenols, polysaccharides and unknown-origin compounds (Grandy et al., 2008, 2009; See Supplemental Tables S1 to S3 for a list of specific compounds and their assigned classes).

DOM-SOM Exchange

To estimate the degree to which O horizon C was transported directly to deep soil horizons vs. exchanging with mineral soil SOM enroute, we compared the chemistry of DOM from E- and B-horizon lysimeters to end members representative of: (1) O-horizon input DOM and (2) the potentially-exchangeable SOM pool. The O-horizon input DOM endmember was simply lysimeter DOM collected from the uppermost lysimeter in each plot and includes DOM contributions from leaf litter in the Oi horizon and leaf litter and fine roots in the Oe horizon (Fig. 1). For the potentially-exchangeable SOM end-member, we conducted a column leaching experiment with intact soil cores to obtain samples that would closely represent the chemical characteristics of potentially-soluble SOM under field conditions (e.g., Zsolnay, 2003). Our goal here was to collect samples to assess the chemical characteristics of the potentially soluble pool of SOM as defined by Sanderman et al. (2008). To accomplish this we collected two sets of five intact cores from both red pine and sugar maple ecosystems: one set containing the entire E horizon and the other set containing the uppermost 45 cm of the B horizon. The E horizon cores were used to collect soluble SOM representative of soil above the middle lysimeter (Fig. 1), whereas the B horizon cores were used to collect soluble SOM representative of soil above the lowermost lysimeter (Fig. 1). Cores were collected on March 10, 2013 and returned on ice to the laboratory. The cores were placed on top of glass funnels packed with glass wool in a 5°C cold room and leached with a 0.01 mM CaCl₂ solution (adjusted to pH 4.1; average pH of lysimeter solutions from our sites), at a rate of 3 to 4 mL min⁻¹ until at least 400 mL of leachate had been collected. Whereas, many factors such as ionic strength, pH, and DOM concentrations will affect the desorption of SOM in situ, for simplicities sake we chose to use dilute CaCl₂ adjusted to the average pH of our lysimeter solutions because numerous studies have suggested this to be appropriate for collecting samples representative of potentially-soluble SOM (e.g., Reemtsma et al., 1999; Zsolnay, 2003; Ros et al., 2010).

Column leachate samples were analyzed for DO¹³C, SUVA₂₅₄ and py-GC/MS as described above for lysimeter samples. The DOM concentrations and volumes from our column leaching experiment were so low that we had to composite all of our column leachate solutions (across both ecosystems, E and B horizons and all replicates) to generate enough C for a single py-GC/MS analysis. We compared SUVA₂₅₄ and py-GC/MS of our mixture samples (E and B lysimeters) to our endmembers graphically. We used the DO¹³C data to quantitatively estimate the proportional contribution of O-horizon C to DOC moving into and out of the B horizon using a two end member mixing model, with DOC draining from the O horizon representing our one end member, and column-extracted DOC representing the other. Because it has been shown that extractable organic C typically is more enriched in δ¹³C than its source SOC (Gauthier et al., 2010; Nakanishi et al., 2012), we also ran our mixing model separately with δ¹³C of bulk SOM as an endmember for purposes of comparison (see Discussion). Statistical sig-

nificance of differences in δ¹³C between end members was first established using simple *t* tests (significant at *P* < 0.01 for both end members in both forest types). We then entered mean values and standard deviations of δ¹³C from these end members, as well as DOC draining the E and B horizons into the isotopic mixing model Iso-Error (Phillips and Gregg, 2001), to estimate means and standard errors for the proportional contribution of surface C to DOC fluxes, while accounting for uncertainty in source-pool enrichment. We initially ran mixing models for each season (spring, summer, autumn) separately; however we observed very little variability in calculated mixing ratios across seasons (CVs <10% across seasons). Mixing ratios reported in Table 3 are based on isotopic values across all seasons. It should be noted that this approach does not account for potential contributions of DOM from fine roots or root exudates in the mineral soil, which likely would have a ¹³C natural abundance similar to that of O horizons (Garten et al., 2000, 2008).

Data Analysis

Differences in DOC fluxes, SUVA₂₅₄ and DO¹³C between ecosystems and across horizons and seasons were analyzed using a linear mixed model, in SAS version 9.3 software (SAS Institute, 2010). We defined seasons as summer (between snowmelt and leaf senescence of deciduous trees), autumn (between leaf senescence and first snow accumulation), winter (between first snow accumulation and the onset of sustained spring snowmelt) and spring (between onset of spring snowmelt and loss of snow cover from all plots). Variance parameters were estimated using the restricted maximum likelihood procedure. Ecosystem type, sampling depth, season and all interactions were included as fixed effects. Observations were blocked by year, plot, and depth, using random group effects (G-side). We used an unstructured covariance model to account for the potential depth autocorrelation at each location. The time autocorrelation was modeled by setting sampling date as a repeated measure (R-side random effect) with a first order autoregressive (AR1) covariance structure. Covariance parameters were allowed to vary according to season. The choice of covariance structure was guided by goodness of fit and the Bayesian information criteria (Schwarz, 1978). Degrees of freedom were calculated using the procedure developed by Kenward

Table 3. Proportional contribution (%) of surface-derived C to total dissolved organic C draining from the E horizon or the second B horizon in red pine and sugar maple stands. Data are means (± 1 SE) calculated from a two end-member mixing model based on δ¹³C natural abundance using δ¹³C of either bulk SOC or column-extracted DOC as a soil end member. Horizon-specific (E or B) end members were used separately for E horizon and B horizon mixing models.

	Bulk SOC End Member	Column Leachate End Member
Red Pine		
E horizon	78 (11.8)	85 (8.7)
B horizon	46 (9.1)	61 (7.7)
Sugar Maple		
E horizon	94 (9.2)	96 (6.5)
B horizon	74 (10.7)	80 (8.3)

and Roger (1997). Model diagnostics (normality, homoskedasticity, goodness of fit) were assessed on the conditional residuals (Haslett and Haslett, 2007). Means were compared using a *t* test with no provision for multiple inferences (Webster, 2007). The significance level of all statistical tests was set to $\alpha = 0.05$.

We compared molecular chemistry (py-GC/MS) of lysimeter DOM and SOM across ecosystems and depths using non-metric multidimensional scaling (NMDS) with random starts to avoid local minima (Oksanen et al., 2010). We used Bray-Curtis distances of the original data and conducted ordinations for: (1) all DOM samples together, (2) all red pine DOM and solid samples together, and (3) all sugar maple DOM and solid samples together. The analyses were conducted using the metaMDS function in the vegan library of R statistical software (R Core Team, 2013).

RESULTS

DOC Fluxes

The 2013 water year was much wetter than normal with 1013 mm of total precipitation, compared to a 30-yr normal of 784 mm. July and August were particularly wet, with 191 and

120 mm of total precipitation, as compared to 30-yr averages of 80 and 89 mm, respectively. The 2014 water year had near-normal total precipitation (835 mm). The sites received abundant snowfall in both years with December-January-February precipitation (liquid equivalent) totaling 202 mm in 2013 and 166 mm in 2014, as compared with the 30-yr average of 142 mm. There were a handful of warm spells with snowmelt and hydrologic flux in December 2012 and January 2013, whereas the winter of 2013 to 2014 was consistently cold, with no snowmelt events occurring between December 2013 and April 2014.

Despite the large differences in O horizon thickness and C stocks between red pine and sugar maple stands (Table 1), there was no significant main effect of ecosystem type (red pine vs. sugar maple) on DOC flux (Fig. 2; $F_{1,395} < 0.00$; $P = 0.999$). We observed consistent reductions in the magnitude of DOC fluxes with depth ($F_{2,12} = 62.45$; $P < 0.001$) with total DOC flux averaging $29 \text{ g C m}^{-2} \text{ y}^{-1}$ ($\pm 2.8 \text{ SD}$) from the O horizon, $18 \text{ g C m}^{-2} \text{ y}^{-1}$ ($\pm 4.4 \text{ SD}$) from the E horizon and $6 \text{ g C m}^{-2} \text{ y}^{-1}$ ($\pm 4.0 \text{ SD}$) from the B horizon. We also observed a strong response of DOC fluxes to seasons ($F_{3,395} = 137.91$; $P < 0.001$), with the highest

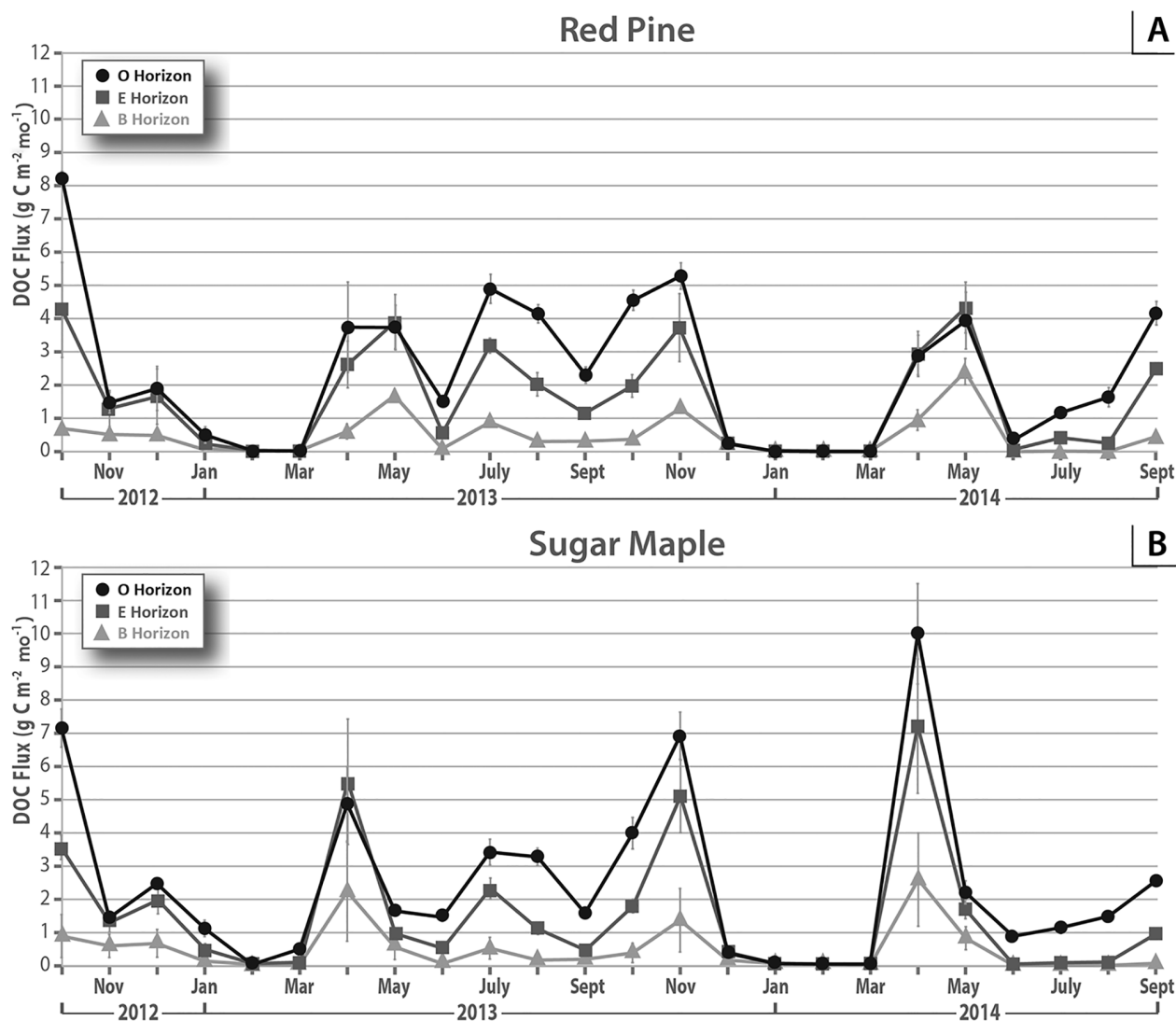


Fig. 2. Monthly dissolved organic carbon (DOC) fluxes by depth from red pine (A) and sugar maple (B) stands from October 2012 through September 2014. Data are means \pm 1 SE of three stands within each ecosystem type.

rates of DOC flux in the spring and autumn, intermediate rates in the summer, and very little DOC flux in the winter. Furthermore, the depth response of DOC flux also varied across seasons, as evidenced by a significant season \times depth interaction ($F_{6,395} = 23.23$; $P < 0.001$). Of particular note, DOC fluxes from the O and E horizons were indistinguishable in the snowmelt-dominated period of spring ($P = 0.285$; Fig. 2), whereas fluxes from E horizons were significantly lower than from O horizons in all other seasons.

DO¹³C and SUVA₂₅₄

Changes in DO¹³C natural abundance with depth, averaged across all sampling dates, are shown in Fig. 3, and compared to $\delta^{13}\text{C}$ of soil organic matter and DOC from the column leaching experiment. Overall, Fig. 3A and B show that $\delta^{13}\text{C}$ of all three sample types: solid SOM, in situ lysimeter DOM, and DOM derived from the column leaching experiment become more enriched in ¹³C with increasing depth. Dissolved organic matter leaving the O horizon in both ecosystems was highly depleted in ¹³C, and very similar to that of fresh litter (Oi horizon). As water percolated through the E horizon, it was only slightly more enriched in ¹³C, but became more enriched after passing through the B horizon. The pattern of enrichment of lysimeter DOM with depth differed between red pine and sugar maple ecosystems, as evidenced by a significant ecosystem \times depth interaction ($F_{2,73} = 4.71$; $P = 0.012$). In red pine ecosystems, DO¹³C of E-horizon samples was significantly greater than that of O-horizon samples ($P = 0.049$), whereas in sugar maple ecosystems there was no change in DO¹³C between O- and E-horizon samples ($P = 0.652$). The DO¹³C of B-horizon samples were significantly greater than those of E-horizon samples beneath both red pine ($P < 0.001$) and sugar maple ($P = 0.010$).

Changes in SUVA₂₅₄ of DOM from in situ lysimeters and column leachate solutions are shown in Fig. 3B and D. We found a significant overall effect of ecosystem type on SUVA₂₅₄ values of lysimeter DOM, with overall greater SUVA₂₅₄ in sugar maple stands than in red pine stands (Fig. 3 B and D; $F_{1,70} = 9.61$; $P = 0.003$). The SUVA₂₅₄ values of lysimeter samples decreased significantly with depth ($F_{2,12} = 10.26$; $P = 0.002$), nonetheless,

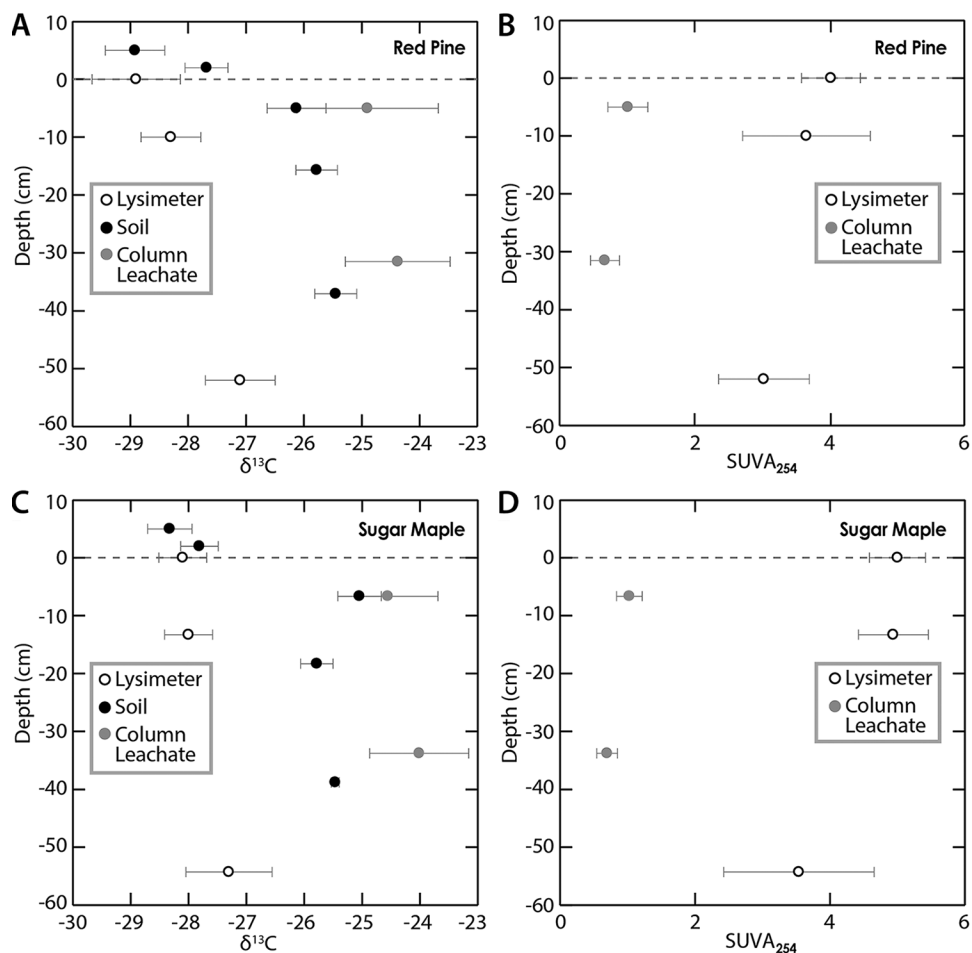


Fig. 3. Patterns of $\delta^{13}\text{C}$ (A and C) and SUVA₂₅₄ (B and D) with soil depth for red pine (A and B) and sugar maple (C and D) stands. $\delta^{13}\text{C}$ data are presented for soil analyzed by genetic horizon sampled at the time of site instrumentation, lysimeter dissolved organic matter (DOM) averaged across sample dates, and DOM from the column-leachate experiment. SUVA₂₅₄ values are presented only for lysimeter DOM and column-leachate solutions. Lysimeter depths represent averages across the three stands within each ecosystem type, whereas soil depths represent horizon midpoints averaged across stands. The dashed vertical line at depth = 0 represents the boundary between organic horizons and mineral soil horizons. Error bars represent 1 SD.

they always remained at least three-fold higher than SUVA₂₅₄ of CaCl₂-extractable DOM from the column leaching experiment. SUVA₂₅₄ of E horizon DOM was indistinguishable from that of incoming O-horizon DOM in both red pine and sugar maple ecosystems ($P = 0.541$), whereas, across both ecosystems, SUVA₂₅₄ of DOM from B-horizon lysimeters was significantly lower than that of O ($P = 0.001$) and E horizons ($P = 0.004$).

Patterns of isotopic and spectroscopic properties of lysimeter DOM with depth are visualized together in Fig. 4. The DOM collected from O-horizon lysimeters, “O-horizon end member,” is rich in aromatics (high SUVA₂₅₄) and depleted in ¹³C as it enters the mineral soil. In contrast, CaCl₂-extractable DOM generated from the column leaching experiment (E- and B-horizon end members) has very low aromaticity and is enriched in ¹³C. As DOM passes through the E horizon in red pine stands, its isotopic and spectroscopic properties become slightly more like that of CaCl₂-extractable DOM, whereas in sugar maple stands there is no shift following percolation through the E horizon.

Application of $\delta^{13}\text{C}$ data in a two end-member mixing model allows for a quantitative estimation of the contribution of surface-derived C to DOC entering and leaving the B horizon (Table 3). Because column-extracted DOC was enriched in ^{13}C relative to bulk SOM, estimates of the contribution of surface-derived C to DOC flux were always greater for calculations using this as an end member. Considering both end members, changes in ^{13}C with depth suggest that approximately 80% of the DOC entering the B horizon in red pine stands is derived from the O horizon, whereas 40 to 60% of lysimeter DOC leaving the lower B horizon is derived from the O horizon. By comparison, O-horizon derived DOC appears to undergo much less exchange with in situ SOM in sugar maple stands, where approximately 95% of the DOC entering the B horizon, and 70 to 80% of that leaving the B horizon, appears to originate from the O horizon.

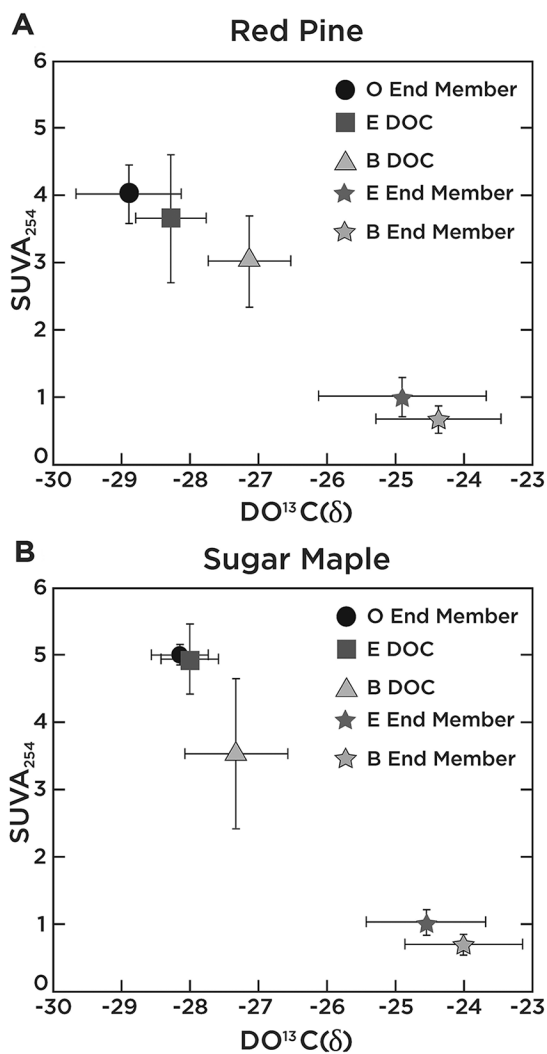


Fig. 4. Biplot of $\delta^{13}\text{C}$ and SUVA_{254} of lysimeter dissolved organic matter (DOM) and column leachate solutions from red pine (A) and sugar maple (B) stands. Lysimeter solutions draining the O horizon are considered the O horizon end member representative of DOM entering the mineral soil, whereas column leachate solutions from E and B horizons are considered end-members for potentially soluble soil organic matter in E and B horizons, respectively. Data are averaged across sample dates and replicate stands; error bars represent 1 SD.

Molecular Chemistry of DOM and SOM

We identified a total of 173 distinct compounds that occurred in at least one of the lysimeter DOM samples analyzed by py-GC/MS (Supplemental Table S1). NMDS analysis of all lysimeter samples produced a two-axis solution with a final stress of 0.094 (Fig. 5A). In red pine stands, O and E horizon lysimeter samples clustered separately, with E-horizon samples occurring between those of O and B horizons. In contrast, O horizon and E-horizon samples from sugar maple stands overlapped broadly, with some separation from the single B horizon sample. Sugar maple DOM samples diverged from red pine DOM samples primarily along Axis 1, whereas across both ecosystems DOM chemistry changed with depth along both Axes 1 and 2.

A total of 63 individual compounds were significantly correlated with NMDS ordination scores (Supplemental Table S1). To visualize how different classes of compounds contribute to the ordination of DOM molecular composition, we created a “box-and whiskers” plot of the distribution of vector direction cosines for these 63 compounds, grouped by compound origin (Fig. 5B). This plot illustrates the distribution of correlations with each NMDS axis, within each compound class. Lignin-derived compounds overwhelmingly exhibit strong negative correlations with Axis 1, whereas N-bearing and protein compounds overwhelmingly exhibit strong positive correlations with Axis 1 (Fig. 5B). Thus increasing values along NMDS Axis 1 appear to indicate decreasing lignin to N ratios. The clear separation of O-horizon DOM from red pine vs. sugar maple stands along Axis 1 indicate that O-horizon DOM from red pine stands had high relative abundance of lignin-derived compounds and low relative abundance of compounds derived from proteins and other N-bearing compounds. Aromatic and lipid compounds overwhelmingly exhibit strong negative correlations with Axis 2, whereas other compounds are highly variable and inconsistent with respect to Axis 2 (Fig. 5B). Changes in molecular composition with increasing soil depth along Axis 2 are associated with an increase in aromatic and lipid compounds.

The py-GC/MS analyses of DOM, litter, soil, and root samples identified 229 distinct compounds in red pine stands (Supplemental Table S2) and 244 compounds in sugar maple stands (Supplemental Table S3). The NMDS analysis produced two-axis solutions with a final stress of 0.055 for red pine (Fig. 6A) and 0.052 for sugar maple (Fig. 6C). In neither ordination was there an indication of chemical similarity between SOM and either O horizon material or root tissues; B horizon SOM was quite similar to lysimeter DOM along Axis 1, but diverged from this material along Axis 2. All fresh plant material, including roots and O horizons, were distinct from lysimeter DOM and soil organic matter along Axis 1. Distributions of variable vector cosines were similar in both ecosystem types (Fig. 6B and D) and show that the change in molecular composition along Axis 1, moving from fresh plant litter to deep soil SOM and/or DOM, is associated with a decline in lignin-derived compounds and an increase in aromatics, lipids and protein-derived compounds. In contrast, differences between solid-phase SOM and lysimeter DOM along

Axis 2 appear to be driven by a greater abundance of lipid, N-bearing and protein-derived compounds in SOM samples. There was less separation between SOM and DOM along Axis 2 in sugar maple stands than for red pine stands.

DOM Exchange and Soil and Solution Characteristics

As indicated by the ^{13}C mixing model, our ^{13}C -SUVA₂₅₄ biplot, and NMDS ordinations of molecular chemistry of lysimeter DOM, we observed greater exchange of DOM with E-horizon SOM in red pine stands compared to sugar maple stands. As an exploratory tool to understand factors that may underlie variability in the degree to which surface-derived DOM exchanged with in situ SOM in transit, we conducted simple linear correlations of our quantitative estimate of the percentage of surface C passing through the E horizon (^{13}C mixing model; Table 3) against a suite of E horizon soil characteristics. We also used sample axis scores of O horizon lysimeter solutions from our NMDS ordination of lysimeter DOM py-GC/MS results (Fig. 5) as measures of chemical characteristics of input DOM. The concentration of imogolite-type minerals (ITM) and ratios of C to pyrophosphate-extractable Fe and Al in E horizons exhibited the strongest correlations with surface C delivery to the B horizon. E horizons with less ITM, and with lower C-saturation of pyrophosphate-extractable Fe and Al, allowed a greater proportion of surface C to pass through unaltered. NMDS Axis 1 sample scores of input DOM were the only other variable that was significantly correlated with the proportion of surface C reaching the B horizon; in this case, higher scores were correlated with a greater proportion of surface C reaching the B horizon (Table 4). The negative correlation between total E horizon C stock and proportion of surface C reaching the B horizon was strong, but not quite statistically significant ($P = 0.083$).

DISCUSSION

Does Surface C Reach the Subsurface Horizons Directly?

We hypothesized that C derived from O horizons should make a greater contribution to DOM entering the B horizon in these coarse-textured Spodosols, when compared to what has been recently reported for other soil types. This hypothesis was borne out as our data provide strong evidence for the direct delivery of O horizon C to depth in these sites. Spectroscopic (SUVA₂₅₄), isotopic ($\delta^{13}\text{C}$) and molecular (py-GC/MS) analyses all consistently indicated that O horizons were the source for the majority of DOM entering B horizons, with little contribution from SOM dissolution en route. Based on our $\delta^{13}\text{C}$ mixing

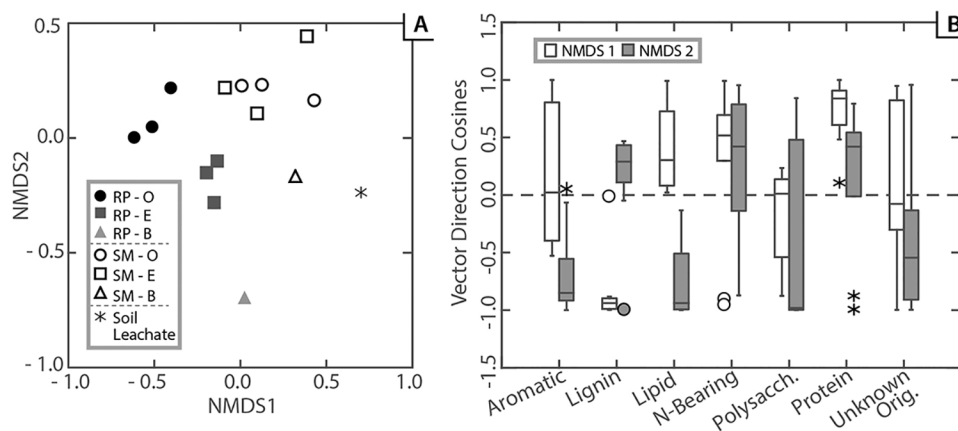


Fig. 5. Non-metric multidimensional scaling ordination of compounds identified by py-GC/MS analysis of lyophilized dissolved organic matter (DOM) samples (A) and "box-and-whiskers" plot showing distribution of vector direction cosines within compound classes (B). In (A), RP is red pine and SM is sugar maple. In (B), the center line represents the median, the box represents the central 50% of values, "whiskers" define the Tukey inner fences, asterisks indicate values outside the inner fences and circles indicate values outside the outer fences.

model, 80 to 95% of the DOM entering B horizons is surface-derived, and even after passing through B horizons, surface-derived C accounts for 40 to 80% of the C in percolating soil water. Thus, as we hypothesized, there is far less exchange with in situ SOM at our sites on Spodosols, than has been reported in C isotope studies in other ecosystems, which consistently report <25% of DOC at depth originating from surface materials. Using additions of ^{14}C -labeled litter, Fröberg et al. (2007) estimated that Oi horizons contributed only 3 to 23% of the DOC at 15 cm and 5 to 23% of the DOC at 70-cm depth in an oak forest in Tennessee. Using ^{13}C -labeled litter manipulations, Hagedorn et al. (2002, 2004) estimated that O horizons constructed from spruce-beech litter contributed only 5 to 8% of total DOC at 5- to 10-cm depth in mesocosms, and Hagedorn et al. (2012) found that O horizons accounted for only 8 to 15% of DOC at 5-cm depth in beech forests on calcareous soils in Switzerland. Sanderman et al. (2008) did not specifically calculate a percentage contribution of surface organic matter, but did cite the decline in SUVA₂₅₄ and enrichment of $\delta^{13}\text{C}$ of DOM as indicators of the exchange of fresh, plant-derived OM for more humified SOM in lysimeters in a redwood (*Sequoia sempervirens*)–Douglas-fir (*Pseudotsuga menziesii*) forest in California. The overall pattern they observed was similar to the results for our red pine sites (Fig. 3A and C), but with a larger apparent contribution from SOM dissolution.

Our use of a ^{13}C mixing model to estimate the contribution of O horizon C to DOM moving through soil is essential for quantitative comparisons with earlier studies that used isotopically-labeled litter; however, it is important to consider any potentially confounding effects of this approach. Of greatest concern is the selection of an appropriate end member that captures the $\delta^{13}\text{C}$ of soil C likely to exchange with DOC in solution. The $\delta^{13}\text{C}$ of bulk SOM is clearly and distinctively more enriched than that of surface O horizons or DOC originating from O horizons. However, not all C in SOM is readily soluble and the pool of potentially-exchangeable SOM is likely to be much smaller (Sanderman et al., 2008) and could differ in $\delta^{13}\text{C}$.

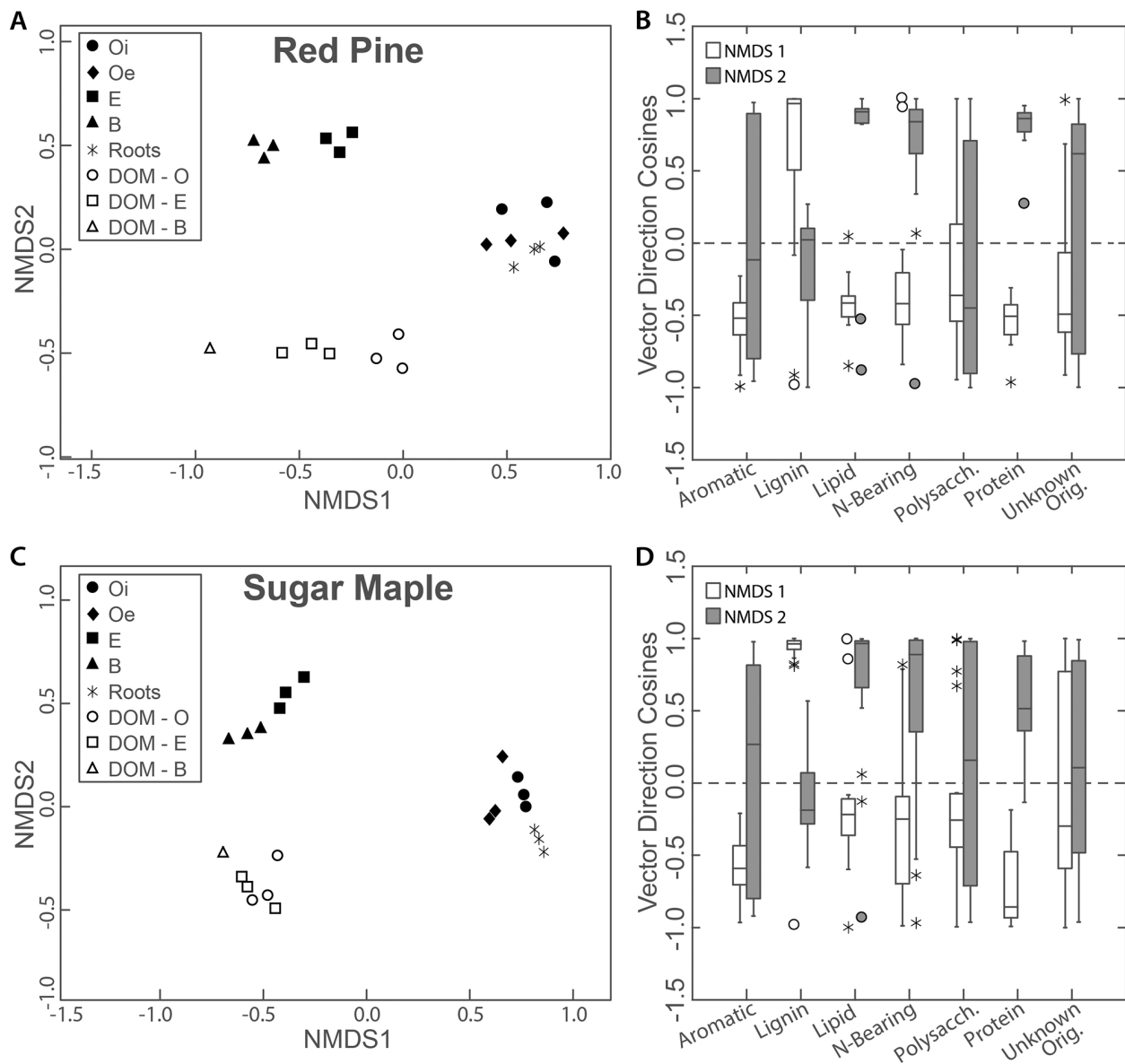


Fig. 6. Non-metric multidimensional scaling ordination of compounds identified by py-GC/MS analysis of lyophilized DOM, soil and root samples from red pine (A) and sugar maple (C) stands. Panels B and D represent “box-and-whiskers” plot showing distribution of vector direction cosines within compound classes for red pine (B) and sugar maple (D) stands. In (B, D), the center line represents the median, the box represents the central 50% of values, “whiskers” define the Tukey inner fences, asterisks indicate values outside the inner fences and circles indicate values outside the outer fences.

Our column-extracted DOC was consistently more enriched in ^{13}C than was bulk SOM, a finding consistent with other studies comparing $\delta^{13}\text{C}$ of water-extractable organic C to its source SOC (e.g., Gauthier et al., 2010; Nakanishi et al., 2012), suggesting that the more hydrophilic fraction of SOM available for exchange with percolating DOM is, in fact, more enriched than bulk SOM. Whereas dilute CaCl_2 extracts have been suggested to be one of the best approaches to generating DOM representative of that which would be soluble in situ (Reemtsma et al., 1999; Zsolnay, 2003; Ros et al., 2010), we acknowledge that this does not truly match the chemistry of percolating soil water, nor can it account for variability in conditions that occur in situ.

Another important caveat to our estimates of the contribution of O horizons to DOM at depth (Table 3) is that we used a two-end-member mixing model that only accounts for potential

contributions from the O horizon and from mineral soil OM; we did not account for the potential contribution of DOM from roots or root exudates within the mineral soil profile. Fine roots are typically enriched in ^{13}C by 1 to 2 per mil compared to fresh leaves (Hobbie and Werner, 2004), but have similar $\delta^{13}\text{C}$ natural abundance as forest floor materials (Garten et al., 2000, 2008). For example, Fahey et al. (2012) found that sugar maple roots from New York had $\delta^{13}\text{C}$ natural abundance between -27.6 and -27.8 , which almost exactly corresponds to what we observed for Oe horizons in our sugar maple stands. Thus, if fine roots in the mineral soil contribute significant quantities of DOM to percolating water, then our mixing model would overestimate the contribution of O horizons to DOM at depth. The fact that py-GC/MS analysis could not differentiate roots from O horizons means that we cannot use this as a tool to evaluate the degree

Table 4. Correlation coefficients and probabilities for simple linear correlations between the proportion of surface-derived C reaching the B horizon as determined by an isotopic mixing model (Table 2) and corresponding soil and input-solution DOM properties ($n = 6$). Statistically significant correlations are indicated by bold text. Plots of all statistically significant correlations can be seen in Supplemental Figure S1.

	<i>r</i>	<i>P</i>
E horizon soil properties		
Horizon thickness, cm†	0.362	0.481
C concentration, %	-0.667	0.148
C Stock, kg m ⁻² ‡	-0.755	0.083
Sand content, %	0.127	0.811
Pyrophosphate Al + Fe (mg kg ⁻¹)	-0.091	0.863
Dithionite Al + Fe (mg kg ⁻¹)	0.179	0.734
Oxalate Al + Fe (mg kg ⁻¹)	-0.509	0.303
C to Al _{py} ratio§	-0.768	0.075
C to Fe _{py} ratio§	-0.756	0.082
IMT, %¶	-0.936	0.036
Input DOM chemistry		
NMDS Axis 1 score	0.842	0.035
NMDS Axis 2 score	0.330	0.523

† Note that E-horizon thickness is equivalent to the second lysimeter depth in Table 1.

‡ Total C in E horizon is calculated from C concentration, bulk density, and horizon depth.

§ Molar ratios of carbon to pyrophosphate-extractable Al or Fe.

¶ IMT, imogolite-type minerals.

to which this overestimation may have occurred. However, we speculate that any overestimation is likely to be minor given that: (1) O-horizon C pools are 3- to 10-fold higher than fine-root C pools in the mineral soil (Tables 1 and 2), and (2) we observed rapid and continual declines in leachate DOM concentrations moving from the base of the O horizon through the mineral soil.

DOM-SOM Exchange in Sugar Maple vs. Red Pine Stands

Carbon derived from the O horizon dominated the DOM delivered to the B horizon in both ecosystem types; however, as we hypothesized, DOM exchange with in situ SOM was much greater in red pine stands (Fig. 4; Fig. 5). Sugar maple stands were particularly noteworthy, in that DOM delivered to the B horizon there was virtually unaltered relative to DOM produced by the O horizon (Fig. 3B; Fig. 4B, Fig. 5A). We used simple correlations with a suite of soil and DOM properties to identify underlying factors driving the delivery of O horizon C to the subsoil (Table 4). Although these correlations are limited by being based on only six data points, they are consistent with a large body of work on DOM transport processes in soil. First, we observed a strong negative correlation between the percentage of ITM in the E horizon and the delivery of O horizon C to the B horizon. This relationship is consistent with the well-established role of short-range order minerals in sorbing DOM from soil solution (Kaiser et al., 1996; Kaiser and Guggenberger, 2000; Farmer and Lumsdon, 2001; Mossin et al., 2002; Kothawala et al., 2009; Kramer et al., 2012). Thus, E horizons that were more strongly leached of ITM allowed a greater proportion of surface

C to pass through unaltered. Similarly, the ratio of E-horizon C to E-horizon pyrophosphate-extractable Al and Fe are indices of the degree of saturation of these metals by organic matter. Thus, E horizons with relatively high C saturation allowed a greater proportion of surface C to pass unaltered.

The strong positive correlation we observed between the NMDS Axis 1 score of input DOM and the delivery of surface carbon to depth is consistent with the understanding that hydrophobic, lignin-derived compounds preferentially sorb and displace hydrophilic, N-rich compounds from soil reactive surfaces (Kaiser and Guggenberger, 2000; Kaiser and Kalbitz 2012; Scott and Rothstein 2014). DOM produced by O horizons in red pine stands had high concentrations of lignin-derived compounds and low concentrations of protein and N-bearing compounds, relative to DOM from sugar maple stands (Fig. 6). Thus, it appears that the chemical composition of DOM produced from forest floors of these species matches well the traditional view of the chemical composition of their respective litter from several studies of litter decomposition, that is, red pine litter has high lignin and low N contents, whereas sugar maple litter has low lignin and high N contents (Aber et al., 1990; Gholz et al., 2000; Parton et al., 2007). Whereas the higher N concentration of sugar maple litter is consistent and unambiguous in the literature, the perceived lower lignin content of sugar maple is unclear as “lignin” values in these studies are all operationally-defined, acid-insoluble fractions that may also include compounds originating from cutin, waxes and condensed tannins (Preston et al., 1997). Our solid-phase py-GC/MS analysis of Oi horizon material indicated a slightly lower content of lignin derived compounds in sugar maple stands ($42 \pm 1.8\%$) compared with red pine stands ($47\% \pm 1.8\%$). Nevertheless, lignin derived compounds made up 26% (± 5.2) of the DOM draining red pine O horizons, compared to only 13% (± 2.5) of the DOM draining sugar maple O horizons.

The negative relationship between total C stock of the E horizon and surface-C delivery was marginally-significant, but makes sense as a potential driving factor, because this parameter represents the amount of in situ C available to exchange with DOC as it percolates through the E horizon. Interestingly, E-horizon thickness, or the path length through which percolating solutions must flow, had no apparent influence on the delivery of surface-derived C to the B horizon, indicating that unaltered surface C can be delivered to great depths, as long as soil and input DOM properties are conducive.

Our correlation analyses suggest that the degree to which surface C pools are “disconnected” from subsurface horizons is determined by the combination of surface horizon mineralogy and the chemical composition of DOM produced in the litter layer, which in turn is determined by characteristics of the dominant vegetation. This interpretation is supported by examining forest and soil characteristics of studies where surface-generated DOM undergoes greater amounts of exchange with in situ SOM than at our sites. These studies have generally been conducted on soils below tree taxa typically associated with high-lignin, poorly decomposing litters, including oak (*Quercus*; Fröberg et al.,

2007; Kramer et al., 2010), beech (*Fagus*; Hagedorn et al., 2002; 2004, 2012), and spruce (*Picea*; Hagedorn et al., 2002, 2004). Although we do not have comparable mineralogical data, soils in these studies appear to have less leached E horizons, higher clay contents, and/or higher SOC concentrations than the soils at our sites. Soil hydrology is likely to be an additional factor contributing to the direct delivery of O horizon C to depth that was not included in our correlation analysis. Kaiser and Kalbitz (2012) specifically note that exchange with SOM is likely to be lower under conditions of rapid percolation. The fact that soils at our sites are largely unstructured and sandy suggests that movement of DOM occurs mainly under conditions of saturated flow, limiting its contact time for exchange with SOM. Thus, we do not view the direct transport of surface C to depth observed in our study as contradictory to the conceptual model put forward by Kaiser and Kalbitz (2012), which emphasizes rapid exchange with in situ SOM. Instead, we argue that the degree to which O horizons deliver C directly to the lower profile varies predictably based on hydrology, vegetation and soil characteristics. Forest ecosystems dominated by species with high-lignin litter, occurring on sites with fine-textured soils, high concentrations of reactive minerals and/or high C contents in surficial horizons should exhibit a strong disconnect between surface C pools and subsurface horizons. In contrast, forest ecosystems like our sugar maple stands that are dominated by species with low-lignin litter, on coarse-textured soils and with surface horizons nearly devoid of reactive minerals and SOM should exhibit strong links between surface C pools and subsurface horizons.

To What Degree Does Surface-Derived DOM Contribute to Subsoil SOM?

Whereas our data clearly demonstrated direct transport of largely-unaltered DOM from surface organic horizons to the subsoil where it was rapidly removed from percolating soil water, it is unclear the degree to which this illuvial transport of surface C contributes to subsoil SOM stocks. The question remains the degree to which this input of O-horizon C contributes directly to stable subsoil SOC, compared with the contribution of in situ root C. Our NMDS ordination, taking into account the entire suite of compounds identified does not provide conclusive evidence for dominance by either pathway. Certainly upper B horizon SOM and eluviating DOM separated from roots and organic horizons (Fig. 6A and C) along NMDS Axis 1. However, because root chemical composition was indistinguishable from that of bulk O horizons (Fig. 6A and C) we cannot conclusively distinguish roots vs. O horizons the source of C for subsoil SOM. However, it is important to note that roots are likely making a significant contribution to DOM produced in the O horizons, as there was intense proliferation of fine roots in Oe horizons of both ecosystem types (Table 2). In fact, two-thirds or more of the fine root biomass we quantified to a 50-cm depth occurred in the O and E horizons, with very low rooting intensity in the subsoil (Table 2). Although our data are not sufficient to quantitatively compare the contribution of in situ root decay vs.

illuvial transport of O-horizon DOM to subsoil SOM stocks, we argue that they are highly suggestive of a significant contribution of illuvial C in these coarse-textured soils.

Another important point to consider is the degree to which subsoil SOM stocks represent direct mineral stabilization of illuviating DOM vs. stabilization of compounds derived from microbial processing of DOM. For example, Marin-Spiotta et al. (2011) and Kramer et al. (2012) used ^{13}C CPMAS NMR to identify sorption of illuvial-transported aromatic compounds in DOM by short-range order minerals in B horizons as the dominant source for subsoil SOM in Hawaiian Andisols. Our py-GC/MS analysis exhibited some similarity to these studies in that negative NMDS Axis 1 scores for upper B horizon SOM and lysimeter DOM indicate a high relative abundance of aromatic compounds in both. However, we found that lysimeter DOM and subsoil SOM diverged markedly along NMDS Axis 2, corresponding to an enrichment of SOM in lipids, proteins and N-bearing compounds (Fig. 6). These data strongly suggest the importance of microbial processing of C inputs to the B horizon (whether from illuvial DOM or in situ roots) as has been well documented in recent investigations into the nature of SOM formation (Miltner et al., 2012; Gleixner, 2013; Kallenbach et al., 2016). However, we cannot quantitatively evaluate the relative importance of direct sorption of illuviating DOM vs. stabilization of microbial products with our data alone.

CONCLUSIONS

Recent studies using isotopically labeled litter additions (Hagedorn et al., 2002, 2004, 2012; Fröberg et al., 2007; Kramer et al., 2010) have concluded that, in some soils, little of the DOM solubilized from surface litter reaches the subsoil directly, implying that O horizon C is largely “disconnected” from mineral soil C stocks (Garten, 2009). Rapid sorption of surface-derived DOM in the uppermost mineral horizons, and concomitant dissolution of in situ SOM, have been proposed to explain this phenomenon (Sanderman et al., 2008; Kaiser and Kalbitz, 2012). However, this view is at odds with traditional pedogenic studies of podzolization, which view surface O horizons as a major source of C for B horizons (Lundström et al., 2000; Sauer et al., 2007). At our study sites, with highly leached E horizons and coarse-textured soils, multiple lines of evidence indicate that DOM entering the B horizon is dominated by unaltered DOM derived directly from the O horizon, pointing to very strong connections between O horizon and mineral soil C pools. Despite the fact that we observed very little exchange as DOM passed through surface horizons, our results over the entire profile are consistent with the “dynamic-exchange” conceptual model of Kaiser and Kalbitz (2012), and, in fact, provide strong empirical support for their conceptual model. Our results confirm their concept that the degree of exchange between DOM and SOM should be controlled by reactive minerals and hydrology, and our results further indicate that litter chemistry may be an additional important driver of exchange. Although we clearly demonstrate significant inputs of O-horizon derived C to the subsoil, we

cannot quantitatively evaluate the importance of illuvial DOM transport vs. in situ root turnover as a source of C to B horizons. Finally, divergence in chemical composition among fresh plant tissues (roots and leaf litter), lysimeter DOM and subsoil SOM (Fig. 6) indicates the importance of microbial processing of C inputs as a source for SOM

ACKNOWLEDGMENTS

We gratefully acknowledge financial support from the National Science Foundation (grant EAR1053373), Michigan State University AgBioResearch, and the USDA National Institute of Food and Agriculture (McIntire Stennis Project MICL06006). We thank Michael Cook, Kent and Melissa Dankenbring, Trevor Kubitskey, Michael Luehmann, Angela Porta, Jay Strahan, and Phil Wernette for their assistance with site instrumentation, field sample collection, graphics preparation and laboratory analysis. We also thank Emily Scott for assistance with multivariate analysis, Stephanie Grand for assistance with data analysis and insights into soil mineralogy, Kathryn Severin for equipment access and Scott Greenwood for the py-GCMS analysis. Finally, we thank Martha Sjogren and the staff at the Hiawatha National Forest, St. Ignace Ranger District for their assistance with identifying and accessing study sites.

SUPPLEMENTAL MATERIAL

Supplemental material is available with the online version of this article. The document contains Table S1, List of compounds identified in dissolved organic matter samples from red pine and sugar maple stands organized by compound class; Table S2, List of compounds identified in dissolved organic matter, soil and root samples from red pine stands organized by compound class; Table S3, List of compounds identified in dissolved organic matter, soil and root samples from sugar maple stands organized by compound class; and Figure S1, Plots of significant correlations between the proportion of surface-derived C reaching the B horizon;

REFERENCES

Aber, J.D., J.M. Melillo, and C.A. McLaugherty. 1990. Predicting long-term patterns of mass loss, nitrogen dynamics, and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. *Can. J. Bot.* 68:2201–2208

Bird, J., and M. Torn. 2006. Fine roots vs. needles: A comparison of C-13 and N-15 dynamics in a ponderosa pine forest soil. *Biogeochemistry* 79:361–382. doi:10.1007/s10533-005-5632-y

Comer, P., D. Albert, H. Wells, B. Hart, J. Raab, D. Price, et al. 1995. Michigan's native landscape, as interpreted from the General Land Office surveys 1816 to 1856. Rep. to the US EPA Water Division and the Wildlife Division, MI Dep. of Natural Resources. Michigan Natural Features Inventory, Lansing, MI. p. 78 and digital map.

Fahey, T.J., K.R. Jacobs, and R.E. Sherman. 2012. Fine root turnover in sugar maple estimated by ¹³C isotope labeling. *Can. J. For. Res.* 42:1792–1795. doi:10.1139/x2012-128

Farmer, V.C., and D.G. Lumsdon. 2001. Interactions of fulvic acid with aluminium and a proto-imogolite sol: The contribution of E-horizon eluates to podzolization. *Eur J Soil Sci* 52:177–188.

Federer, C. 2013. BROOK90: A simulation model for evaporation, soil water, and streamflow. Ver. 4.6. Computer freeware and documentation. <http://www.ecoshift.net/brook/brook90.htm> (Accessed 15 Nov. 2013).

Fröberg, M., P. Jardine, P. Hanson, C. Swanston, D. Todd, J. Tarver, and C. Garten. 2007. Low dissolved organic carbon input from fresh litter to deep mineral soils. *Soil Sci. Soc. Am. J.* 71:347–354. doi:10.2136/sssaj2006.0188

Garten, C.T., L.W. Cooper, W.M. Post, and P. Hanson. 2000. Climate controls on forest soil C isotope ratios in the southern Appalachian Mountains. *Ecology* 81:1108–1119. doi:10.1890/0012-9658(2000)081[1108:CCO FSC]2.0.CO;2

Garten, C.T., P.J. Hanson, D.E. Todd, B.B. Lu, D.J. Brice, K. Lajtha, and R.H. Michener. 2008. Natural ¹⁵N and ¹³C abundance as indicators of forest nitrogen status and soil carbon dynamics. In: R.H. Michener, and K. Lajtha, editors, *Stable isotopes in ecology and environmental science*. 2nd ed. Blackwell Science, Oxford, UK. p. 61–82.

Garten, C. 2009. A disconnect between O horizon and mineral soil carbon—implications for soil C sequestration. *Acta Oecologica-International Journal of Ecology* 35:218–226. doi:10.1016/j.actao.2008.10.004

Gauthier, A., P. Amiotte-Suchet, P.N. Nelson, J. Lévêque, B. Zeller, and C. Hénault. 2010. Dynamics of the water extractable organic carbon pool during mineralization in soils from a Douglas fir plantation and an oak-beech forest—an incubation experiment. *Plant Soil* 330:465–479. doi:10.1007/s11104-009-0220-x

Gholz, H.L., D.A. Wedin, S.M. Smitherman, M.E. Harmon, and J.W. Parton. 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: Toward a global model of decomposition. *Glob. Change Biol.* 6:751–765. doi:10.1046/j.1365-2486.2000.00349.x

Gleixner, G. 2013. Soil organic matter dynamics: A biological perspective derived from the use of compound-specific isotopes studies. *Ecol. Res.* 28:683–695. doi:10.1007/s11284-012-1022-9

Grandy, A., M. Strickland, C. Lauber, M. Bradford, and N. Fierer. 2009. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. *Geoderma* 150:278–286. doi:10.1016/j.geoderma.2009.02.007

Grandy, A., R. Sinsabaugh, J. Neff, M. Stursova, and D. Zak. 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. *Biogeochemistry* 91:37–49. doi:10.1007/s10533-008-9257-9

Hagedorn, F., A. Kammer, M. Schmidt, and C. Goodale. 2012. Nitrogen addition alters mineralization dynamics of ¹³C-depleted leaf and twig litter and reduces leaching of older DOC from mineral soil. *Glob. Change Biol.* 18:1412–1427. doi:10.1111/j.1365-2486.2011.02603.x

Hagedorn, F., M. Saurer, and P. Blaser. 2004. A C-13 tracer study to identify the origin of dissolved organic carbon in forested mineral soils. *Eur. J. Soil Sci.* 55:91–100. doi:10.1046/j.1365-2389.2003.00578.x

Hagedorn, F., P. Blaser, and R. Siegwolf. 2002. Elevated atmospheric CO₂ and increased N deposition effects on dissolved organic carbon-clues from delta C-13 signature. *Soil Biol. Biochem.* 34:355–366. doi:10.1016/S0038-0717(01)00191-2

Harrison, R., P. Footen, and B. Strahm. 2011. Deep soil horizons: Contribution and importance to soil carbon pools and in assessing whole-ecosystem response to management and global change. *For. Sci.* 57:67–76.

Haslett, J., and S. Haslett. 2007. The three basic types of residuals for a linear model. *Int. Stat. Rev.* 75:1–24. doi:10.1111/j.1751-5823.2006.00001.x

Hobbie, E., and R.A. Werner. 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: A review and synthesis. *New Phytol.* 161:371–385. doi:10.1111/j.1469-8137.2004.00970.x

Jobbagy, E., and R. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* 10:423–436. doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

Kaiser, K., and G. Guggenberger. 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. *Org. Geochem.* 31:711–725. doi:10.1016/S0146-6380(00)00046-2

Kaiser, K., and K. Kalbitz. 2012. Cycling downwards- dissolved organic matter in soils. *Soil Biol. Biochem.* 52:29–32. doi:10.1016/j.soilbio.2012.04.002

Kaiser, K., G. Guggenberger, and W. Zech. 1996. Sorption of DOM and DOM fractions to forest soils. *Geoderma* 74:281–303. doi:10.1016/S0016-7061(96)00071-7

Kalbitz, K., D. Schwesig, J. Rethemeyer, and E. Matzner. 2005. Stabilization of dissolved organic matter by sorption to the mineral soil. *Soil Biol. Biochem.* 37:1319–1331. doi:10.1016/j.soilbio.2004.11.028

Kallenbach, C.M., S.D. Frey, and A.S. Grandy. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nat. Commun.* 7:13630. doi:10.1038/ncomms13630

Kenward, M., and J. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53:983–997. doi:10.2307/2533558

Kothawala, D., T. Moore, and W. Hendershot. 2009. Soil properties controlling the adsorption of dissolved organic carbon to mineral soils. *Soil Sci. Soc. Am. J.* 73:1831–1842. doi:10.2136/sssaj2008.0254

- Kramer, C., S. Trumbore, M. Fröberg, L. Dozal, D. Zhang, X. Xu, et al. 2010. Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. *Soil Biol. Biochem.* 42:1028–1037. doi:10.1016/j.soilbio.2010.02.021
- Kramer, M., J. Sanderman, O. Chadwick, J. Chorover, and P. Vitousek. 2012. Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. *Glob. Change Biol.* 18:2594–2605. doi:10.1111/j.1365-2486.2012.02681.x
- Lundström, U., N. van Breemen, and D. Bain. 2000. The podzolization process: A review. *Geoderma* 94:91–107. doi:10.1016/S0016-7061(99)00036-1
- MacDonald, J.N., S. Bélanger, F. Sauvé, W. Courchesne, and W. Hendershot. 2008. Collection and characterization of soil solutions. In: M. Carter, and E. Gregorich, editors, *Soil sampling and methods of analysis*. 2nd ed. CRC Press, Boca Raton, FL. p. 179–196.
- Marin-Spiotta, E., O. Chadwick, M. Kramer, and M. Carbone. 2011. Carbon delivery to deep mineral horizons in Hawaiian rain forest soils. *J. Geophys. Res. Biogeosci.* 116.
- McKeague, J. 1967. An evaluation of 0.1 M pyrophosphate and pyrophosphate-dithionite in comparison with oxylate as extractants of accumulation products in podzols and some other soils. *Can. J. Soil Sci.* 47:95–99. doi:10.4141/cjss67-017
- Miller, B.A., and R.J. Schaetzl. 2012. Precision of soil particle size analysis using laser diffractometry. *Soil Sci. Soc. Am. J.* 76:1719–1727.
- Miltner, A., P. Bombach, B. Schmidt-Brücken, and M. Kästner. 2012. SOM genesis: Microbial biomass as a significant source. *Biogeochemistry* 111:41–55. doi:10.1007/s10533-011-9658-z
- Mizota, C., and L. Van Reeuwijk. 1989. Clay mineralogy and chemistry of soils formed in volcanic material in diverse climatic regions. *ISM Monograph*. Institute of Statistical Mathematics, Japan.
- Mossin, L., M. Mortensen, and P. Nornberg. 2002. Imogolite related to podzolization processes in Danish podzols. *Geoderma* 109:103–116. doi:10.1016/S0016-7061(02)00145-3
- Nakanishi, T., M. Atarashi-Andoh, J. Koarashi, Y. Saito-Kokubu, and K. Hirai. 2012. Carbon isotopes of water-extractable organic carbon in a depth profile of forest soil imply a dynamic relationship with soil carbon. *Eur. J. Soil Sci.* 63:495–500. doi:10.1111/j.1365-2389.2012.01465.x
- Oksanen, J., F.G. Blanchet, R. Kindt, P. Legendre, B. O'Hara, G.L. Simpson, et al. 2010. *Vegan: Community ecology package*. R Package Version 1.17–3. R Foundation for Statistical Computing, Vienna, Austria.
- Parton, W., W.L. Silver, I.C. Burke, L. Grassens, M.E. Harmon, W.S. Currie, J.Y. King, E.C. Adair, L.A. Brandt, S.C. Hart, and B. Fash. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* 315:361–364. doi:10.1126/science.1134853
- Phillips, D., and J. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171–179. doi:10.1007/s004420000578
- Preston, C.M., J.A. Trofymow, B.G. Sayer, and J.N. Niu. 1997. C-13 nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies. *Can. J. Bot.* 75:1601–1613. doi:10.1139/b97-872
- Price, S., M. Bradford, and M. Ashton. 2012. Characterizing organic carbon stocks and flows in forest soils. In: M.S. Ashton, M.L. Tyrrell, D. Spalding, and B. Gentry, editors, *Managing forest carbon in a changing climate*. Springer, the Netherlands. p. 7–30. doi:10.1007/978-94-007-2232-3_2
- R Core Team. 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rasse, D., C. Rumpel, and M. Dignac. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilization. *Plant Soil* 269:341–356. doi:10.1007/s11104-004-0907-y
- Reemtsma, T., A. Bredow, and M. Gehring. 1999. The nature and kinetics of organic matter release from soil by salt solutions. *Eur. J. Soil Sci.* 50:53–64. doi:10.1046/j.1365-2389.1999.00212.x
- Ros, G., E. Hoffland, and E. Temminghoff. 2010. Dynamics of dissolved and extractable organic nitrogen upon soil amendment with crop residues. *Soil Biol. Biochem.* 42:2094–2101. doi:10.1016/j.soilbio.2010.08.004
- Rumpel, C., and I. Kogel-Knabner. 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant Soil* 338:143–158. doi:10.1007/s11104-010-0391-5
- Sanderman, J., J. Baldock, and R. Amundson. 2008. Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Biogeochemistry* 89:181–198. doi:10.1007/s10533-008-9211-x
- SAS Institute. 2010. *SAS version 9.3 software*. SAS Inst., Inc., Cary, NC.
- Sauer, D., H. Sponagel, M. Sommer, L. Giani, R. Jahn, and K. Stahr. 2007. Review article. Podzol: Soil of the year 2007: A review on its genesis, occurrence, and functions. *J. Plant Nutr. Soil Sci.* 170:581–597. doi:10.1002/jpln.200700135
- Sayer, E. 2006. Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Biol. Rev. Camb. Philos. Soc.* 81:1–31. doi:10.1017/S1464793105006846
- Schaetzl, R., M. Luehmann, and D. Rothstein. 2015. Pulses of podzolization: The relative importance of spring snowmelt, summer storms, and fall rains on sodosol development. *Soil Sci. Soc. Am. J.* 79:117–131. doi:10.2136/sssaj2014.06.0239
- Schoeneberger, P.J., D. Wysocki, and E. Benham, and Soil Survey Staff. 2012. *Field book for describing and sampling soils*, Version 3.0. NRCS National Soil Survey Center, Lincoln, NE.
- Schwarz, G. 1978. Estimating the dimension of a model. *Ann. Stat.* 6:461–464. doi:10.1214/aos/1176344136
- Scott, E., and D. Rothstein. 2014. The dynamic exchange of dissolved organic matter percolating through six diverse soils. *Soil Biol. Biochem.* 69:83–92. doi:10.1016/j.soilbio.2013.10.052
- Webster, R. 2007. Analysis of variance, inference, multiple comparisons and sampling effects in soil research. *Eur. J. Soil Sci.* 58:74–82.
- Weihermüller, L., J. Siemens, M. Deurer, S. Knoblauch, H. Rupp, A. Göttlein, and T. Pütz. 2007. In situ soil water extraction: A review. *J. Environ. Qual.* 36:1735–1748.
- Weishaar, J., G. Aiken, B. Bergamaschi, M. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* 37:4702–4708. doi:10.1021/es030360x
- Wilson, L., G. Everett, and S. Cullen. 1995. *Handbook of vadose zone characterization and monitoring*. Lewis Publishers, Boca Raton, FL.
- Zsolnay, A. 2003. Dissolved organic matter: Artefacts, definitions, and functions. *Geoderma* 113:187–209. doi:10.1016/S0016-7061(02)00361-0